



Research Journal of **Microbiology**

ISSN 1816-4935



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Growth Promotional Potential of *Pseudomonas fluorescens* FPD-10 and its Interaction with *Bradyrhizobium* sp.

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Abstract: A number of rhizosphere bacterial strains belonging to fluorescent pseudomonads have been used as seed inoculants to promote plant growth and increase yields. A pot experiment was devised as completely randomized factorial design with two main factors to study the effect of application of seven levels of *Pseudomonas fluorescens* FPD-10 with *Bradyrhizobium* strains NC-92 and SSP-24 under preincubated and co inoculated conditions (as first main factor) in different time intervals (as the second main factor) with 3 replicates. For each measured response variable (root and shoot biomass, nodule number and biomass and nitrogen content) an analysis of variance (ANOVA) was performed. So the ability of *Pseudomonas fluorescens* FPD-10 to promote plant growth was assessed under greenhouse conditions using JL-24 variety of peanut as test crop. The inoculation of FPD-10 significantly increased the root dry weight when compared to control. The interaction between FPD-10 and *Bradyrhizobium* strains NC-92 and SSP-24 were studied under preincubated and coinoculated conditions. The preincubated treatments gave better results than the coinoculated treatments with respect to root dry weight, shoot dry weight, nodule number, nodule dry weight and per cent N content of shoot.

Key words: *Pseudomonas fluorescens*, plant growth promoting rhizobacteria, coinoculation, preincubation

INTRODUCTION

The free living soil bacteria inhabiting the root and rhizosphere and having the ability to increase plant growth are usually referred as plant growth promoting rhizobacteria (PGPR). They include a number of different bacteria such as *Azotobacter*, *Azospirillum*, *Pseudomonads*, *Acetobacter*, *Burkholderia* and *Bacillus* (Glick, 1995). The mechanisms by which PGPR enhances the plant growth and crop yields are not fully understood. The possible explanations given by workers worldwide include the reduction of ethylene level in developing plant roots by their ability to produce ACC deaminase thereby increasing the root length and growth (Glick *et al.*, 1995; Li *et al.*, 2000; Penrose and Glick, 2001), production of growth promoting substances like Indole Acetic Acid (IAA), gibberellic acid and cytokinins (Frankenberger and Arshad, 1995; Patten and Glick, 2002), symbiotic nitrogen fixation (Kennedy *et al.*, 1997), antagonism against phytopathogenic microorganisms (Pal *et al.*, 2001)

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and solubilization of mineral phosphates and mineralization of other nutrients (Defreitas *et al.*, 1997). Among them the strains belonging to fluorescent pseudomonads, have been used as seed inoculants on crop plants to promote plant growth and increase crop yields (Kloepper *et al.*, 1980; Suslow and Schroth, 1982). Subsequent reports suggested that PGPR could exert positive effects on diverse hosts like bean (Anderson and Guerra, 1985), cotton (Sakthivel *et al.*, 1986), soybean (Polonenko *et al.*, 1987), peanut (Dey *et al.*, 2004), sugarbeet (Cakmakci *et al.*, 2006) and maize (Shaharoon *et al.*, 2006). Recently a review was published detailing the history of PGPR discovery and also indicating the progress in understanding each of the PGPR groups (Gray and Smith, 2005). The present study was undertaken with the objective of assessing the growth promoting potential of *Pseudomonas fluorescens* FPD-10 and its interaction with *Bradyrhizobium* sp. in peanut crop.

MATERIALS AND METHODS

Greenhouse Evaluation of Bacterial Strains

The ability of bacterial strains to promote plant growth was assessed under greenhouse conditions in the Department of Agricultural Microbiology, UAS, Dharwad using JL-24 variety of peanut as the test crop. The experiment was conducted in pots (15×15 cm) in a factorial design with a basic design which was completely randomized. Medium black clay soil obtained from E-block, Main Research station, U.A.S. Dharwad was used in the study. Peanut seeds were sown after bacterizing them with respective bacterial cultures. In a pot two seeds were sown and after germination only one seedling was retained in each pot. The pots were maintained in the greenhouse for a period of 49 days and the sampling was done at 35, 42 and 49 days after inoculation. Each pot comprised of an experimental unit and the treatments (inoculation×time) were assigned randomly to the pots. The experiment consisted of treatments (with 8 levels) and days after inoculation (independent pots for 3 levels of 35, 42 and 49 DAI) as two main factors having eight treatments which were replicated three times. The treatment combinations were

- Uninoculated control
- *Pseudomonas fluorescens* FPD-10 single inoculation
- *Bradyrhizobium* sp. NC-92 single inoculation
- *Bradyrhizobium* sp. SSP-24 single inoculation
- *Pseudomonas fluorescens* FPD-10 + *Bradyrhizobium* sp. NC-92 preincubated
- *Pseudomonas fluorescens* FPD-10 + *Bradyrhizobium* sp. SSP-24 preincubated
- *Pseudomonas fluorescens* FPD-10 + *Bradyrhizobium* sp. NC-92 coinoculated
- *Pseudomonas fluorescens* FPD-10 + *Bradyrhizobium* sp. SSP-24 coinoculated

The population of *Pseudomonas fluorescens* FPD-10, *Bradyrhizobium* sp. NC-92 and *Bradyrhizobium* sp. SSP-24 at the time of sowing was 9.5×10^8 , 6.2×10^8 and 6.5×10^8 mL⁻¹, respectively. The treatment and the interaction between *Pseudomonas fluorescens* FPD-10 and strains of *Bradyrhizobium* sp. were studied in two ways. In one set, called preincubation, equal suspensions of both bacteria were mixed and incubated together for five hours on a rotary shaker at 50 rpm and 28°C as per protocol of Nishijima *et al.* (1988) and then used for inoculation. For coinoculation, the individually grown cultures were mixed in equal proportion just before sowing and then applied. In both preincubated and coinoculated treatments, the seeds were bacterized with 1 mL of bacterial suspension. All the bacterial cultures used in the study were obtained from the Department of Agricultural Microbiology, UAS, Dharwad.

Statistical Analysis

In this experiment main effects of treatment and day after inoculation were assigned according to completely randomized factorial design with three replicates. Sampling of seedlings was done at 35, 42 and 49 days after Inoculation (DAI). The root and shoot dry weight (after drying in an oven at 65°C till constant weight), nodule number, dry weight of nodules and shoot N concentration through Kjeldahl method (Jackson, 1973) were determined. The differences between each treatment and the non-inoculated control was calculated and for each variable an analysis of variance and comparison of means using SAS procedure PROC GLM (for General Linear Model) and MEAN statement (SAS, 1999) was performed to assess the main effect of treatment, the main effect of days after inoculation and the interaction of the two.

RESULTS

The main effects (treatment and days after inoculation) and their interaction were significant for all response variables (Table 1). It can be observed from the Fig. 1a that, the inoculation of FPD-10 significantly ($p<0.05$) increased root dry weight at 42 and 49 days after inoculation (DAI). At 35, 42 and 49 DAI, the preincubation of FPD-10 with *Bradyrhizobium* sp. SSP-24 and NC-92 was found to be significantly superior to the coinoculation method (Fig. 1a). Differences between root dry weight after both 42 and 49 DAI and 35 DAI was statistically significant ($p<0.05$) which signified a growing trend in root dry weight over time. Preincubation of FPD-10 with *Bradyrhizobium* sp. SSP-24 and NC-92 increased the shoot dry weight at 35, 42 and 49 DAI significantly ($p<0.05$) compared to other treatments. The pattern of change of shoot dry weight was different from that of root dry weight as it was increased at 42 DAI and dropped at 49 DAI (Fig. 1b). On all days of sampling the preincubated treatments were significantly superior over coinoculated treatments (Fig. 1b). Among individual strains, it was noted that FPD-10 was able to improve the root growth, shoot growth, nodule number and N content in shoot than NC-92 and SSP-24.

Preincubation of FPD-10 with *Bradyrhizobium* sp. SSP-24 and NC-92 recorded nodule number and nodule dry weight that were significantly ($p<0.05$) higher than other treatments at all stages of sampling (Fig. 1c and d). A generally significant increase in the dry weight and number of nodules at 49 DAI was observed in all treatments as compared to 35 and 42 DAI (Fig. 1c and d). With regard to the N content in shoot, the treatment receiving the preincubated mixture of FPD-10

Table 1: Factorial analysis of variance of root dry weight, shoot dry weight, nodule number, nodule dry weight and nitrogen content in peanut plants inoculated with *Pseudomonas fluorescens* and strains of *Bradyrhizobium*

Response variable	Source	df	Type III SS	Mean square	F-value	Pr>F
Root dry weight	TREAT	6	0.06794622	0.01132437	101.48	<0.0001
	DAI	2	0.23780822	0.11890411	1065.57	<0.0001
	TREAT*DAI	12	0.03511644	0.00292637	26.22	<0.0001
Shoot dry weight	TREAT	6	0.28307343	0.0471789	462.61	<0.0001
	DAI	2	0.05104289	0.02552144	250.25	<0.0001
	TREAT*DAI	12	0.048158	0.00401317	39.35	<0.0001
Nodule number	TREAT	6	3905.333333	650.888889	80.4	<0.0001
	DAI	2	7965.809524	3982.904762	492.01	<0.0001
	TREAT*DAI	12	1330.857143	110.904762	13.7	<0.0001
Nodule dry weight	TREAT	6	45171.5875	7528.5979	799.57	<0.0001
	DAI	2	116019.4613	58009.7306	6160.89	<0.0001
	TREAT*DAI	12	13448.6186	1120.7182	119.03	<0.0001
Nitrogen content	TREAT	6	10.86872698	1.8114545	126.12	<0.0001
	DAI	2	0.41082222	0.20541111	14.3	<0.0001
	TREAT*DAI	12	1.83637778	0.15303148	10.65	<0.0001

Results in terms of significance probabilities ($P>F$). There were 7 treatments (TREAT) and 3 days after inoculation (DAI) as main effects and the interaction between the main effects (TREAT*DAI) were also tested. DF = Degrees of Freedom

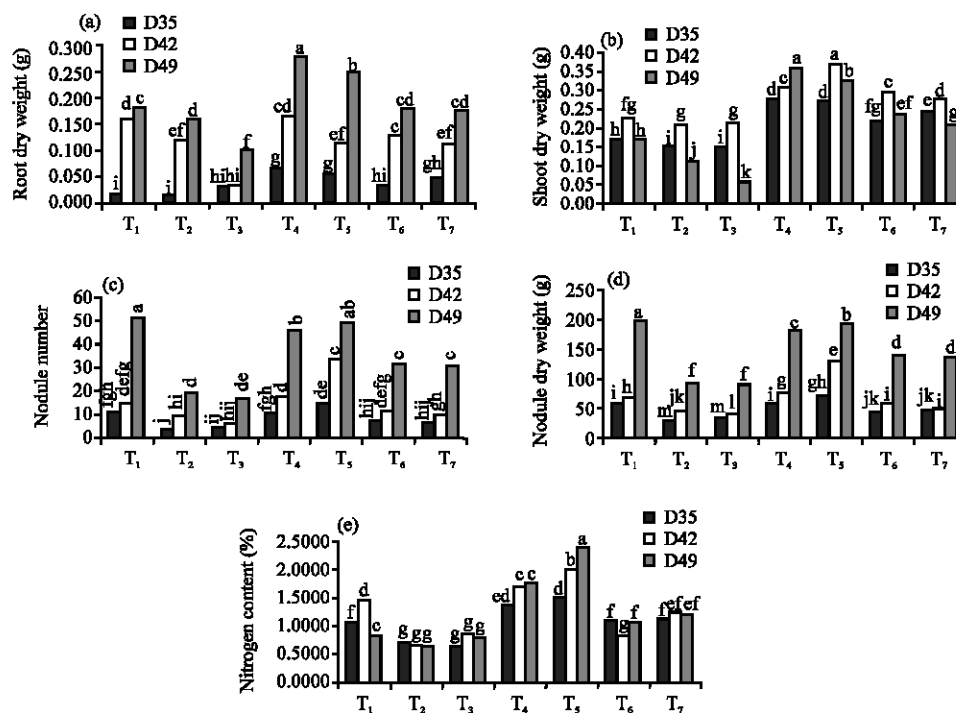


Fig. 1: Mean profiles of a) Root dry weight, b) Shoot dry weight, c) Nodule number, d) Nodule dry weight and e) Nitrogen content over a period of 14 days after inoculation at three time points, 35, 42 and 49 dai. The comparison of mean was done using Duncan's multiple range test at $p < 0.05$ and bars with same letter do not show significant difference in means of the respecting treatments. Means are deducted from un-inoculated control. The X-axis and Y-axis in each graph show treatments and measured response variable respectively. Tags of X-axis are as follows: T₁ = FPD-10 single inoculation T₂ = NC-92 single inoculation, T₃ = SSP-24 single inoculation, T₄ = FPD-10 + NC-92 preincubated, T₅ = FPD-10 + SSP-24 preincubated, T₆ = FPD-10 + NC-92 coinoculated, T₇ = FPD-10 + SSP-24 coinoculated, D35, D42 and D49 = 35, 42 and 49 days after inoculation, respectively

and SSP-24 (7.00%) at 49 DAI recorded the highest percent N content followed by the same treatment at 42 DAI and preincubated treatment of FPD-10 and NC-92 (6.44%) at 42 DAI (Fig. 1e). The nitrogen content of shoot in plants showed an increasing trend as the days progressed due to inoculation of preincubated mixture of FPD-10 and SSP-24 and preincubated treatment of FPD-10 and NC-92 while in other treatments no particular trend was observed (Fig. 1e).

DISCUSSION

Fluorescent pseudomonads are a group of PGPR which are present in the rhizosphere of crop plants and they aggressively colonize the roots. These bacteria are antagonistic to deleterious bacteria, phytopathogenic fungi, produce plant growth promoting substances and increase the crop yields. In the present study the growth promotional potential of *Pseudomonas fluorescens* FPD-10 and its interaction with *Bradyrhizobium* sp. in peanut was studied and the results are discussed in this chapter. The inoculation of FPD-10 was found to have a positive influence in improving the root biomass, shoot growth, nodulation and shoot N concentration. The increased root and shoot growth

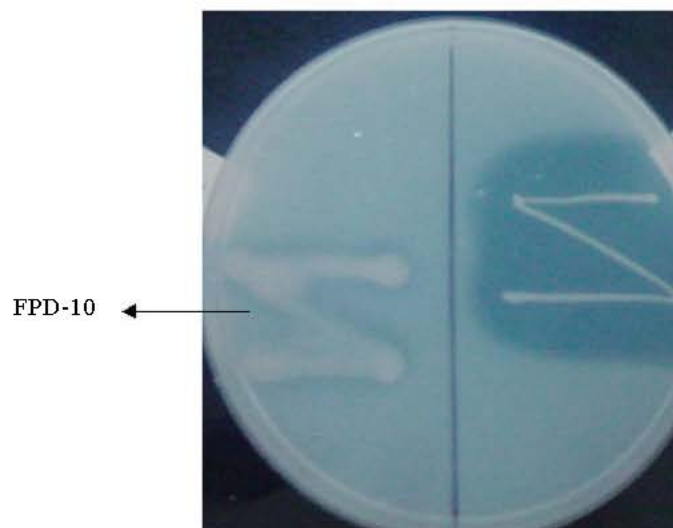


Fig. 2: Growth of *Pseudomonas fluorescens* FPD-10 on hydroxyapatite medium (Slimy appearance of FPD-10 can be noted)

could be attributed to the ability of this strain to solubilize phosphate and due to the release of growth promoting substances like auxins and cytokinins. The inoculation of mineral phosphate solubilizers has increased the total biomass and grain yield in chickpea (Alagawadi and Gaur, 1988) and other leguminous crops (Gaur, 1990). The inoculation of *Pseudomonas fluorescens* isolates PGPR 1, PGPR 2 and PGPR 4 to peanut significantly increased the root length, nodule number, nodule dry weight, pod yield, nitrogen and phosphorus contents in soil (Dey *et al.*, 2004). Phytohormones such as auxins and cytokinins are known to cause enhanced cell division and root development (Arshad and Frankenberger, 1993). In earlier studies *Pseudomonas fluorescens* has been shown to have the capacity to produce cytokinins (Neito and Frankenberger, 1989) and indole acetic acid (Dey *et al.*, 2004) and improve plant growth.

Inoculation of FPD-10 resulted in a tremendous increase in nodule number at later stages of growth. It has been reported earlier that exopolysaccharides are known to influence legume root infection and nodulation (Chen *et al.*, 1985; Leigh *et al.*, 1988). In the present study FPD-10 was able to produce exopolysaccharides as evidenced by high amounts of slime it produced *in vitro* (Fig. 2). It is suspected that the production of exopolysaccharides by FPD-10 resulted in more number of nodules and nodule dry weight than SSP-24 and NC-92. Similarly the shoot N concentration was also much higher in FPD-10 inoculated plants when compared to SSP-24 and NC-92. Increased nodule number is known to result in increased N fixation and N uptake amongst leguminous oil seeds (Joshi *et al.*, 1990).

In the initial stages the differences between dual inoculations and respective single inoculation of *Bradyrhizobium* was not very large with respect to improvement in shoot growth, root growth and nodule number. But towards later stages of growth (42 and 49 DAI) the increased improvement in growth was observed between coinoculated or preincubated treatments over single inoculation of *Bradyrhizobium* strains. The inoculation of FPD-10 with NC-92 and SSP-24 both by addition of preincubated mixture or coinoculation significantly improved per cent N accumulated over the respective NC-92 and SSP-24 single inoculations which indicated an overall net positive effect of FPD-10 on N uptake. Thus it can be inferred that the interaction of the strains vary according to the stages of growth. Positive interactions of *Pseudomonas fluorescens* and *Bradyrhizobium* have been

reported in soybean (Polonenko *et al.*, 1987). Combined inoculation of *Bradyrhizobium* and *Pseudomonas* sp. was found to increase the dry matter accumulation, increased nodule number, dry weight of nodule and nutrient uptake in chickpea (Alagawadi and Gaur, 1988). In another study coinoculation of *Bacillus polymyxa* and *Rhizobium elti* stimulated *Rhizobium elti* populations and nodulation in the rhizosphere of *Phaseolus vulgaris* (Petersen *et al.*, 1996). Field experimentations by dual inoculation are very much required to confirm the positive interaction between FPD-10 and strains of *Bradyrhizobium* on peanut plant.

Preincubation of *Bradyrhizobium japonicum* with *Pseudomonas fluorescens* before inoculation was shown to increase the level of nodulation in soybean plants (Nishijima *et al.*, 1988). Hence an attempt was made in this study to assess the differences between inoculation of preincubated mixture and coinoculation of *Pseudomonas fluorescens* FPD-10 and *Bradyrhizobium* strains on the growth parameters of groundnut. A comparison between coinoculated and preincubated treatments revealed that the preincubated treatments had a significantly higher impact on the root growth, shoot growth, nodule number, nodule dry weight and shoot N content over coinoculated treatments. In our study the preincubation of FPD-10 and *Bradyrhizobium* strains before inoculation resulted in significantly higher nodulation than coinoculated treatments. Similar results of enhanced nodulation were observed when soybean was inoculated with a preincubated mixture of *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* (Nishijima *et al.*, 1988). They suggested that the enhanced nodulation of soybean by *Bradyrhizobium japonicum* in presence of *Pseudomonas fluorescens* could be due to a substance produced by *Pseudomonas fluorescens* SSJ2. In studies conducted earlier interaction between plant growth promoting rhizobacteria and *Bradyrhizobium* increased nodulation and nitrogen fixation in soybean and *Lupinus albus* (Dashti *et al.*, 1998; Garcia *et al.*, 2004). Increased nodulation and N fixation by FPD-10 or FPD-10 with *Bradyrhizobium* strains than individual inoculation of *Bradyrhizobium* in the present study suggests two possibilities. One could be that FPD-10 was able to improve nodulation and N fixation of *Bradyrhizobium* by production of growth promoting substances and the other reason could be that FPD-10 strain is capable of nitrogen fixation. Studies related to the capability of *Pseudomonas fluorescens* and *Pseudomonas* sp. to fix nitrogen has been reported earlier (Gowda and Watanabe, 1985; Chan *et al.*, 1994). However there are suggestions that the contribution of bacterially fixed nitrogen to plants is minimal and that enhanced growth by an inoculated plant does not necessarily mean that the bacteria associated with the roots do fix nitrogen or pass the products of nitrogen fixation to the plant (James and Olivares, 1997). This hypothesis can be confirmed by conducting acetylene reduction assay of the strain, *in vitro* nitrogen fixation and refined plant N uptake analysis. It would be advisable to check whether FPD-10 has the capability to produce growth promoting substances or not. The positive interactions between FPD-10 and strains of *Bradyrhizobium* on peanut growth can be confirmed and used for commercial exploitation only after conducting field trials.

ACKNOWLEDGMENT

Authors are thankful to KSDA, Bangalore, India for providing required funds to carry out this trial. Financial support of Shiraz. University Iran to the 3rd Author is appreciated.

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